

II. REMARKS

Formal Matters

Claims 1-21, 23, and 61-90 are pending after entry of the amendments set forth herein.

Claims 1-21 and 23 were examined. Claims 1-4, 6-8, 10, 12-21 and 23 were rejected. Claims 5, 9, and 11 were objected to.

Claims 1, 5, 9, 11, and 15 are amended. The amendment to claim 1 is merely editorial in nature; as such, no new matter is added. Claims 5, 9, and 11 are amended to be in independent form. Support for the amendments to claims 5, 9, and 11 is found throughout the specification, by the claims from which each claim depends, and Example 1. As such, no new matter is added. Claim 15 is amended solely in the interest of expediting prosecution, and this amendment is not to be construed as acquiescence to any objection or rejection of any claim. Support for the amendments to claim 15 is found in the specification, in particular at the following exemplary locations: paragraphs 90 through 92. Accordingly, no new matter is added by these amendments.

Claims 61-90 are added. Support for new claims 61-83 is found in the claims as originally filed, and throughout the specification, including the following exemplary locations: paragraphs 71 and 79; Example 1, e.g., paragraph 0086; and paragraphs 0019 and 0060.

New claims 61-66 depend from prior pending claims and recite that the prokaryotic host cell is *E. coli*. New claims 67 and 68 depend from prior pending claims and recite that a particular enzyme is an *E. coli* or a *Saccharomyces cerevisiae* enzyme.

New independent claim 69 recites that the transformed host microorganism comprises at least two operons heterologous to the host microorganism. New claims 70-83 depend, directly or indirectly, from claim 69. Applicants note that U.S. Patent Publication No. 2003/0033626, cited in the December 2, 2005 Information Disclosure Statement, includes prophetic examples relating to vectors including a single operon.

Support for new claims 84-90 is found in the claims as originally filed, and throughout the specification. Claims 84 and 85 are supported by the specification at, e.g., paragraphs 49-50 and Examples 1-2. Claims 86-87 are supported by the specification at, e.g., paragraphs 24 and 74, Figure 3 and Example 4.

No new matter is added by new claims 61-90.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

PTO SB-08A form

Applicants respectfully request that the Examiner initial and return the PTO SB-08A form submitted with the Information Disclosure Statement filed on December 22, 2005 and the Supplemental Information Disclosure Statement filed concurrently with this Amendment.

Withdrawal of previous rejections

Applicants note with gratitude that the following rejections, raised in the July 28, 2005 Office Action, have been withdrawn: 1) rejection of claims 1-4, 6, 8, 10, 12-21, and 23 under 35 U.S.C. §112, first paragraph, “written description”; and 2) rejection of claims 1-4, 6-8, 10, 21-21 and 23 under 35 U.S.C. §103(a) over Takagi in view of the cited secondary references.

Rejection under 35 U.S.C. §112, second paragraph

Claims 15-21 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite.

The Office Action stated that claim 15 recites “wherein the isopentenyl pyrophosphate is further modified to provide an isoprenoid”; and stated that the metes and bounds of the claim are not clear “since the claim does not specifically recite how the isopentenyl pyrophosphate is modified to provide the recited products.” Office Action, page 2. Applicants respectfully traverse the rejection. There is no requirement under 35 U.S.C. §112, second paragraph, that the claim recite how the isopentenyl pyrophosphate (IPP) is modified to provide an isoprenoid product.

Requirements under 35 U.S.C. §112, second paragraph

The claims must set out the subject matter **with a reasonable degree of clarity and particularity**. As set forth in MPEP §2173.02, in reviewing a claim for compliance with 35 U.S.C. §112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope, in other words, whether the scope of the claim is clear to a person of ordinary skill in the relevant art.

As set forth in MPEP §2173.02, definiteness of claim language must be analyzed in light of:

- a) the content of the disclosure of the patent application;
- b) the teachings of the prior art; and
- c) the claim interpretation that would be given by one possessing the ordinary level of skill in the art at the time the invention was made.

Claims 15-21 meet the requirements of 35 U.S.C. §112, second paragraph

Applicants submit that when the above factors are taken into consideration, claims 15-21 meet the requirements of 35 U.S.C. §112, second paragraph.

The instant specification provides working examples of generating three different isoprenoid compounds from IPP. Examples 3 and 4 describe production of the carotenoid lycopene; Example 5 describes production of the sesquiterpene amorpho-4,11-diene; and Example 5 describes production of the diterpene casbene.

Given the teachings in the art and the disclosure of the instant application, the scope of the claims would be clear to a person of ordinary skill in the art. Accordingly, claims 15-21 are in compliance with the requirements of 35 U.S.C. §112, second paragraph.

Nevertheless, to expedite allowance, claim 15 is amended to recite that the IPP is modified by the action of isopentenyl pyrophosphate isomerase and one or more polyprenyl pyrophosphate synthases to provide the recited products. Support for this amendment is found at paragraphs 0090 – 0092 of the specification. The Examiner is respectfully urged to withdraw the rejection.

Conclusion as to the rejection under 35 U.S.C. §112, second paragraph

Applicants submit that the rejection of claims 15-21 under 35 U.S.C. §112, second paragraph, has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejections under 35 U.S.C. §103(a)

Claims 1, 3, 4, 6-8, 10, 12-14, and 23 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Takagi et al. ((2000) *J. Bacteriol.* 182:4153-4157; “Takagi”) in view of Hiser et al. ((1994) *J. Biol. Chem.* 269:31383-31389) and Accession L20428; “Hiser”) and Wang et al. (Accession No. AF119715; “Wang”). Claim 2 was rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Takagi in view of Hiser and Wang, and further in view of Balbas et al. ((1996) *Gene* 172:65-69; “Balbas”). Claims 15-21 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Takagi in view of Hiser and Wang, and further in view of Fujisaki et al. ((1986) *J. Biochem.* 99:1327-1337; “Fujisaki”).

Standards for establishing a prima facie case of obviousness

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 21 USPQ2d 1941 (Fed. Cir. 1992). Second, there must be a reasonable expectation of success. *In re Merck & Co., Inc.*, 231 USPQ 375 (Fed. Cir. 1986). Finally, the prior art reference, or references when combined, must teach or suggest all the claim limitations. *In re Royka*, 180 USPQ 580 (CCPA 1974). If any one of these three criteria is not met, a *prima facie* case of obviousness has not been established.

Claims 1, 3, 4, 6-8, 10, 12-14, and 23 over Takagi in view of Hiser and Wang

The Office Action stated that: a) Takagi teaches a method comprising the steps of culturing a transformed *E. coli* JM109 strain harboring a gene cluster for the mevalonate pathway that is contained in the plasmid pUMV19, where the gene cluster encodes mevalonate kinase, diphosphomevalonate decarboxylase, phosphomevalonate kinase, and MHG-CoA synthase; b) Takagi does not teach that the JM109 strain is transformed with a nucleic acid encoding an acetoacetyl-CoA thiolase which condenses two molecules of acetyl-CoA to acetoacetyl-CoA; and an IPP isomerase; c) Hiser teaches a nucleic acid from *S. cerevisiae* encoding an acetoacetyl-CoA thiolase that condenses two molecules of acetyl-CoA to acetoacetyl-CoA; and d) Wang teaches a nucleic acid encoding an IPP isomerase. The Office Action concluded that it would have been obvious to modify the method of Takagi such that the JM109 strain is transformed with the nucleic acid encoding an acetoacetyl-CoA thiolase which condenses two molecules of acetyl-CoA to acetoacetyl-CoA as taught by Hiser and the nucleic acid taught by Wang encoding an IPP isomerase. Applicants respectfully traverse the rejection.

Applicants respectfully assert that the rejection is in error and should be withdrawn because, at least, (i) Takagi does not teach or suggest a process for forming IPP that involves “condensing two molecules of acetyl-CoA to acetoacetyl-CoA,” which is required by each independent claim of the pending claims, and in fact, Takagi teaches away from this; (ii) Takagi does not provide any motivation to generate IPP in a genetically modified host cell, using a mevalonate pathway that involves condensing two molecules of acetyl-CoA to generate acetoacetyl-CoA, and in fact teaches away from this; and (iii) Hiser does not suggest that the acetoacetyl-CoA thiolase encoded by the *Saccharomyces cerevisiae*

erg10 gene would or even could be used in prokaryotic hosts for the purpose of making IPP by a mevalonate pathway. To the contrary, Hiser also teaches away from this claimed aspect.

There is no motivation, either in the references themselves or in the knowledge generally available, to modify the primary reference or to combine reference teachings.

The Office Action stated that one of ordinary skill in the art at the time the invention was made would have been motivated to modify the method of Takagi “in order to have a beneficial culturing method that produces isopentenyl pyrophosphate (IPP).” Office Action, page 4. The Office is reminded that, as set forth in MPEP §2143.01, **the cited art must suggest the desirability of the claimed invention**. The cited art has made no such suggestion.

The initial burden is on the Office to provide some suggestion of the desirability of doing what is claimed. To support the conclusion that the claimed invention directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the Office must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references.¹

Takagi is not concerned with providing a beneficial culturing method that produces IPP via the mevalonate pathway as instantly claimed. Instead, Takagi is concerned with generating “a useful strain for preparing mutants possessing a metabolic block(s) in the nonmevalonate pathway.” *See, e.g.,* Takagi, page 4156, column 2, under “Conclusion.” Takagi, page 4153, bridging paragraph, columns 1 and 2 further evidences that Takagi’s primary concern was whether a strain in which mutations in the DXP pathway (the “non-mevalonate pathway”) could be made which would still yield an IPP level sufficient to maintain cell viability via a partial mevalonate pathway (as depicted in Figure 1A of Takagi). As such, Takagi was concerned with studying the DXP pathway. There is no motivation in Takagi to improve the production of IPP in the strain constructed.

Takagi indicates that the goal of generating a strain for preparing mutants in the DXP pathway was achieved: Takagi states, “the labeling patterns of ubiquinone in this experiment thus proved the

¹ *Ex parte Clapp*, 227 USPQ 972, 973 (BPAI 1985)

operation of the mevalonate pathway in *E. coli* JM109 (pUMV19).”² Takagi, page 4155, column 2.

Thus, Takagi does not provide any motivation to “modify the method of Takagi such that the JM109 strain is transformed with the nucleic acid encoding an acetoacetyl-CoA thiolase which *condenses two molecules of acetyl-CoA to acetoacetyl-CoA*.”

Takagi states that *E. coli* possesses two sequences of acetoacetyl-CoA synthase, citing GenBank Accession Nos. P76461 and Q46936.³ GenBank Accession No. P76461 provides the amino acid sequence of acetoacetyl-CoA thiolase. Takagi states that the role of the acetoacetyl-CoA thiolase depicted in GenBank Accession No. P76461 is “undefined.” Takagi, page 4155, bridging paragraph, columns 1 and 2. Takagi goes on to conclude that the labeling data indicate that acetoacetyl-CoA is generated by the process depicted in Figure 1A of Takagi, i.e., a pathway involving carboxylation of acetyl-CoA to generate malonyl-CoA, and subsequent acetylation of malonyl-CoA to generate acetoacetyl-CoA.

Because Takagi indicates that the mevalonate pathway involves condensing acetyl-CoA and malonyl-CoA to generate acetoacetyl-CoA, and because Takagi indicates that the role of acetoacetyl-CoA thiolase is undefined, Takagi provides no motivation to generate IPP via a mevalonate pathway that involves *condensing two molecules of acetyl-CoA*. In fact, Takagi teaches away from the claimed method by suggesting a starting point of acetyl Co-A processed with acetyl-CoA carboxylase. Indeed, Takagi suggests against the use of **any** exogenous genes involved in processing acetyl Co-A to acetoacetyl-CoA in *E. Coli*, including the two enzymes of Takagi’s choice (i.e., CoA carboxylase and acetoacetyl-CoA synthase)⁴, not to mention the enzyme acetoacetyl-CoA thiolase employed by the instant invention.

Hiser does not cure the deficiency of Takagi. Hiser is not concerned with providing “a beneficial culturing method that produces IPP.” Instead, Hiser is focused on characterizing genes and gene

² The “mevalonate pathway” according to Takagi is as depicted in Figure 1A of Takagi, and involves a two-step process for generating acetoacetyl-CoA, via a malonyl-CoA intermediate.

³ GenBank Accession No. Q46936 provides the amino acid sequence of transcriptional activator protein lysR. Transcriptional activator protein lysR is apparently not involved in the synthesis of acetoacetyl-CoA, and thus does not appear to be relevant to a discussion of acetoacetyl-CoA production.

⁴ Takagi, page 4156, bridging paragraph, columns 1 and 2: “Since wild-type *E. coli* has acetyl-CoA carboxylase and acetoacetyl-CoA synthase genes in the genome, it is not necessary to introduce these enzymes genes for the operation of the mevalonate pathway in *E. coli*.”

products in yeast, a eukaryote. Hiser reports that the *ERG10* locus of *S. cerevisiae* contains a single gene that is the structural gene for acetoacetyl-CoA thiolase. There is no suggestion or motivation in Hiser, to modify the reference to use the gene that codes for acetoacetyl-CoA thiolase in the synthesis of IPP via a mevalonate pathway in a host prokaryotic microorganism that does not normally synthesize IPP, wherein the pathway comprises condensing two molecules of acetyl-CoA to acetoacetyl-CoA as instantly claimed. Hiser neither discloses nor suggests modifying the method of Takagi such that the JM109 strain is transformed with the nucleic acid encoding an acetoacetyl-CoA thiolase which condenses two molecules of acetyl-CoA to acetoacetyl-CoA. Indeed, Hiser states that acetoacetyl-CoA thiolase in bacteria functions in the biosynthesis of poly- β -hydroxybutyrate, a storage molecule. Hiser, page 21383, column 2, first full paragraph. Such disclosure does not provide motivation to use the acetoacetyl-CoA thiolase as claimed. In fact, Hiser teaches away from a combination with Takagi by discussing only a part of a pathway in a eukaryote, not as a prokaryote that does not normally synthesize IPP through the mevalonate pathway.

Wang does not cure the deficiency of Takagi. The isopentenyl pyrophosphate isomerase for which the Office Action cites Wang does not form acetoacetyl-CoA from acetyl-CoA. Instead, as disclosed in the specification at paragraph 0075, such enzymes are useful when “it is desired to retain isopentenyl pyrophosphate in the host microorganism for further biochemical processing, . . . the heterologous nucleic acid sequences . . . [can] include a DNA fragment coding for an enzyme capable of converting isopentenyl pyrophosphate to dimethylallyl pyrophosphate . . . a suitable isomerase will catalyze the conversion of isopentenyl pyrophosphate into dimethylallyl pyrophosphate.”

Therefore, when reviewed as a whole, there is not only a lack of suggestion to combine the references in the manner claimed but also a suggestion against the claimed combination.

Claim 2 over Takagi in view of Hiser and Wang, and further in view of Balbas

The Office Action stated that: a) claim 2 is unpatentable over Takagi, in view of Hiser and Wang as applied to claims 1, 3, 4, 6-8, 10, 12-14, and 23; b) Balbas teaches a method for integration of cloned DNA into the *E. coli* chromosome. The Office Action stated that it would have been obvious to modify the method of Takagi such that the nucleic acids encoding the mevalonate pathway are integrated into the *E. coli* strain. Applicants respectfully traverse the rejection.

As discussed above, the combination of Takagi, Hiser, and Wang fails to disclose or suggest the claimed invention as recited in claim 1, from which claim 2 depends. Balbas merely discusses a family of plasmids for chromosomal integration of cloned DNA into the *E. coli* genome. Balbas thus does not cure the deficiency of Takagi, Hiser, or Wang. Accordingly, Takagi, Hiser, and Wang, alone or in combination with Balbas, cannot render claim 2 obvious.

Claims 15-21 over Takagi in view of Hiser and Wang, and further in view of Fujisaki

The Office Action stated that Fujisaki teaches that isopentenyl pyrophosphate isomerase, farnesyl pyrophosphate synthetase, octaprenyl pyrophosphate synthetase, and undecanyl pyrophosphate synthetase are four enzymes in *E. coli* that in combination ensure the *in vivo* synthesis of long-chain isoprenoids in *E. coli*. The Office Action stated that it would have been obvious to modify the method of Takagi such that the isoprenoid precursor is reacted with the enzymes taught by Fujisaki for the purpose of having a method that produces isoprenoids. Applicants respectfully traverse the rejection.

As discussed above, the combination of Takagi, Hiser, and Wang fails to teach or suggest all of the steps and elements of the claimed invention. Fujisaki, by merely discussing enzymes that are involved in *in vivo* synthesis of long-chain isoprenoids, does not cure the deficiency of Takagi, Hiser, and Wang. Accordingly, Takagi, Hiser, and Wang, alone or in combination with Fujisaki, cannot render claims 15-21 obvious.

Conclusion as to the rejections under 35 U.S.C. §103(a)

Applicants submit that the rejection of the claims discussed above under 35 U.S.C. §103(a) has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Allowable subject matter

The Examiner has objected to claims 5, 9, and 11 as depending from a rejected base claim. The Office Action stated that claims 5, 9, and 11 would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claims 5, 9, and 11 have been rewritten in independent form to include all of the limitation of the base claim and all intervening claims and, therefore, should be allowable.

Additional citations

A Supplemental Information Disclosure Statement accompanies the instant response. Applicants respectfully request that the Examiner initial and return the PTO SB-08A form submitted with the Information Disclosure Statement filed herewith, thereby indicating that the references cited therein have been reviewed and made of record.

For completeness of the record, the Applicants are citing PCT patent publication No. WO 02/10398 and EP 1360300, which are believed to be counterparts of the US patent application publication Nos. 2003/0033626; 2004/0194162; and 2005/24107, made of record in the Information Disclosure Statement filed on December 22, 2005. Related subject matter by the same authors is disclosed in Hahn *et al.*, Jan. 2001, *J. Bact.* 183(1):1-11, made of record in the Information Disclosure State filed March 25, 2002. Applicants are also citing US Patent No. 6,989,257, which is the issued counterpart of US patent application publication No. 2003/0148416, made of record in the Information Disclosure Statement filed on August 15, 2003, as well as related US patent application publication No. 2005/0266518 and the EP and PCT counterpart applications (EP1392824 and WO 02/099095). Applicants are also citing U.S. Patent No. 6,916,972, which relates to cloning of plant mevalonate pathway-related genes. For a complete listing of references, please see PTO SB-08A form.

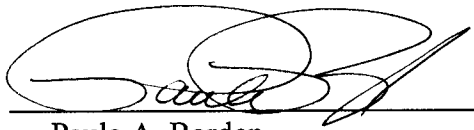
III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number BERK-036.

Respectfully submitted,
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